Diversity of fungi from mangrove sediments of Goa, India, obtained by metagenomic analysis using Illumina sequencing

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Abstract

The fungal composition, abundance and diversity of the mangrove sediments from the Mandovi and Zuari estuaries, Goa, using paired-end Illumina sequencing, hitherto unexplored by a metagenomic approach, indicated that though the types of fungal phyla were similar between the two sediments, the abundance of the species was significantly different between them (p value < 0.005). Basidiomycota and Ascomycota were the two major phyla which were sub-divided into eighteen classes, families, orders, genera and species and one unassigned group in both the sediments. The top five classes observed were *Agaricomycetes*, *Sordariomycetes*, *Saccharomycetes*, *Dothideomycetes* and *Eurotiomycetes* from both the sediments. The diversity analysis based on the observed fungal species richness (Chao 1 for Mandovi were 614 and 714.7 while for Zuari were 665 and 771.2) revealed that Zuari sediment was taxonomically rich, indicating these to be potent candidates for bioremediation and a rich repository for biotechnologically important fungi. This is a first report on diversity of fungi from mangrove sediments of Goa using metagenomic studies.

Keywords Fungal diversity · Goa · Illumina · Mangrove · Metagenomic

Genome reports

The mangrove ecosystem that covers a quarter of the world's tropical coastlines is a major driver for transferring organic matter from land to oceans and is a niche for wide spectrum of microbial diversity (Simoes et al. 2015). Yet it is one of the most threatened ecosystems. Therefore, researchers have comprehensively studied their microbial composition and activities with a view to protect, restore and better understand this ecosystem functioning. However, studies have generally focused on bacterial diversity, and very few studies have investigated the fungal communities from this ecosystem (Simoes et al. 2015). But fungi form an important member of the microbial consortia in mangrove sediments that not only take part in biogeochemical cycling but are also important producers of commercially important enzymes (Simoes et al. 2015). In addition, arbuscular mycorrhizae

(phylum *Glomeromycota*) form symbiotic relationships with over 80% of vascular plants including mangroves (French 2017). In return for carbon, these fungi improve plant health and tolerance to environmental stress (French 2017). Till date, examination of fungal diversity and activities from mangroves of Goa relied on culture-based studies on individual and/or specific groups of fungi (Verma et al. 2010; Nazareth et al. 2011, 2012; Dastager et al. 2012; Devi et al. 2012; Nayak et al. 2012; Bicholkar and Nazareth 2015). However, in the modern era of scientific advancements, 'omic' technologies are emerging as potential tools to ensure holistic insight into environmental systems, thereby leading to in-depth understanding of the complex mechanisms in an ecosystem (Sharma and Lal 2017). It forms a radical tool that helps to detect microbes and to decipher their full biological activities within the environmental niche which otherwise would have remained unnoticed due to our inability of mimicking their growth conditions under laboratory set-ups. There are very few reports on diversity analysis of fungi from mangroves around the world using metagenomic approach (Thompson et al. 2013; Simoes et al. 2015) and most of these studies targeted the analysis of specific fungal genes related to synthesis of various biotechnologically important enzymes (Peng et al. 2012; Thompson et al. 2013).

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However, metagenomic sequencing of native soil communities from mangroves, suggests that they support a variety of mycorrhizal species with potential agricultural, medical and biotechnological applications (French 2017). Moreover, although metagenomic study has been undertaken to study bacterial diversity of mangroves in Goa, using pyrosequencing (Fernandes et al. 2014) and a more in-depth process of Illumina sequencing (Halder and Nazareth 2018), this study forms a first report on fungal diversity from mangrove ecosystem of Goa using next-generation sequencing (NGS). The limitation for metagenomic analysis of fungal communities might be due to the difficulty in isolation of the fungal DNA from sediments and hence an integrated lysis procedure is required to obtain sufficient amount of fungal DNA (Jiang et al. 2011). However, an in-depth phylogenetic analysis of fungal community from the mangrove sediments can be beneficial for developing a better perception on the ecological role of fungi in the mangrove ecosystem.

Therefore, the objective of this study was to investigate the overall fungal composition, abundance and diversity in the mangrove sediments of Mandovi and Zuari estuaries of Goa using internal transcribed spacer (ITS) sequencing on Illumina MiSeq platform, a method that is significantly reliable in its efficiency to discriminate between similar soil samples in comparison to other next-generation sequencing technologies (Habtom et al. 2017).

The investigations of this study were carried out on samples obtained from mangrove forests of Ribandar (15.4993°N and 73.8684°E) and Cortalim (15.4058°N and 73.9286°E) along Mandovi (M) and Zuari (Z) estuaries, respectively, during May 2017. *Avicennia officinalis* and *Avicennia alba* are the two true mangrove species reported from these two places constituting 80% and 40% of the total mangrove vegetation in Ribandar and Cortalim, respectively; *Sonneratia alba* (15%) and *Acanthus ilicifolius* (5%) are also

found at Ribandar mangroves, while Cortalim mangroves are also rich in *Avicennia officinalis* and *Avicennia alba*, *Acanthus ilicifolius*, *Aegiceras corniculatum* and *Excoecaria agallocha* (<http://www.forest.goa.gov.in/mgr/> accessed on 16th March, 2019). A total of six cores of sediment samples from the intertidal zone, three cores from each site, were collected from a depth of 2 cm. The sediment cores were collected using a soil-hole borer from at a depth of 2 cm, transferred to sterile plastic containers with the help of sterile spatula, and were taken to the laboratories for analysis within 1 h of collection. The samples were stored at -20°C before being sent for NGS analyses to Xcelris laboratories, Ahmedabad. The procedure involved preparation of ITS2-amplicon libraries using Nextera XT Index Kit (Illumina Inc) with the adaptor-ligated primers (forward: GCATCGATGAAGAACGCA GC and reverse: TCCTCCGCTTATTGATATGC). The kit is notably successful in rapid library preparation from low-input DNA (1 ng) where DNA is simultaneously fragmented and tagged with sequencing adapters in a single-tube enzymatic reaction. After obtaining the Qubit concentration for the library and the mean peak size from Bioanalyzer profile, the library was loaded onto Illumina MiSeq platform at appropriate concentration (10–20 pM) for cluster generation and sequencing. The Illumina reads obtained from MiSeq platform were stitched using FLASH assembler; filtered using usearch61 algorithm and then taxonomically classified at 97% sequence similarity using UCLUST algorithm (Edgar 2010). The metagenomic sequences were deposited at Sequence Read Archive (SRA of National Center for Biotechnology Information, NCBI) to obtain accession numbers (Table 1).

A total of 1,869,222 and 1,646,924 paired-sequence reads were obtained for A and B which were stitched to produce 716,293 and 724,591 reads. After removal of chimeric sequences, 1,155,904 quality-filtered sequences

Table 1 “Minimal information about metagenomic sequence” (MiMS) for the NGS sequence data submitted to SRA database of GenBank in NCBI for Mandovi and Zuari mangrove sediment

Structured comment name	Mandovi mangrove sediment (A)	Zuari mangrove sediment (B)
Submitted_to_insd	Sequence Read Archive (SRA); accession number: SRX3203327	Sequence Read Archive (SRA); accession number: SRX3203326
Investigation_type	Diversity of fungus in Mandovi mangrove sediment, Goa	Diversity of fungus in Zuari mangrove sediment, Goa
Project_name	Application of metagenomic technique for isolation of microbes from mangroves for potential use in biofertilization and bioremediation	Application of metagenomic technique for isolation of microbes from mangroves for potential use in biofertilization and bioremediation
Lat_lon	15.4993°N and 73.8684°E	15.4058°N and 73.9286°E
Geo_loc_name	Mandovi mangrove estuary, Goa	Zuari mangrove estuary, Goa
Collection_date	2016-04-01T:10:30:10	2016-04-02T:11:45:10
Biome	Mangrove	Mangrove
Feature	Coastal	Coastal
Material	Sediment	Sediment
Seq_meth	Illumina MiSeq	Illumina MiSeq

were obtained which were used for operational taxonomic unit (OTU) generation. Representative set of OTUs consisting of 4140 sequences was prepared from the obtained OTUs. After the removal of spurious and single sequence containing OTUs, a total of 811 OTUs with no singletons, that is, OTUs with ≥ 2 sequences, were obtained and were used for further analysis.

Basidiomycota followed by Ascomycota were the two major phyla identified in A (80.86% and 7.57%) and B (81.7% and 7.2%). Zygomycota was observed as a minor phylum in both A (2%) and B (3%). Other sequences which could not be resolved taxonomically were grouped under unassigned phyla, the latter constituting 9.64% and 8.1% in A and B, respectively. Our findings corroborate earlier findings on mangrove sediments of Goa and others which showed these phyla as the major identified phyla (Verma et al. 2010; Nazareth et al. 2012; Nogueira-Melo et al. 2014; Bicholkar and Nazareth 2015). It is noteworthy to mention that these groups of fungi, isolated from mangroves of Goa, have been reported to be potent candidates used for bioremediation due to their ability of producing laccase (Verma et al. 2010) and metal sorption and removal from solution (Gazem and Nazareth 2012, 2013 Nazareth et al. 2012; Bicholkar and Nazareth 2015).

In both A and B, the two phyla were sub-divided into eighteen classes, families, orders, genera and species. The rest of the sequences which could not be resolved taxonomically were grouped under unassigned class, class, family, order, genus and species, respectively, in A and B (p value < 0.005).

The top five classes observed were Agaricomycetes (75.48% in A and 76.36% in B; p value < 0.005), Sordariomycetes (2.46% in A and 1.37% in B; p value < 0.005), Saccharomycetes (2.37% in A and 2.48% in B; p value < 0.005), Dothideomycetes (2.00% in A and 2.5% in B; p value < 0.005) and Eurotiomycetes (0.53% in A and 0.63% in B) which were sub-divided into Auriculariales (41.81% in A and 42.26% in B; p value < 0.005), Agaricales (33.52% in A and 33.9% in B; p value < 0.005), Saccharomycetales (2.37% in A and 2.48% in B; p value < 0.005), Hypocreales (2.23% in A and 1.32% in B; p value < 0.005) and Pleosporales (1.89% in A and 1.62% in B; p value < 0.005) orders. Finer resolution identified Auriculariaceae (41.8% in A and 42.26% in B; p value < 0.005), Entolomataceae (1.8% in A and 12.45% in B; p value < 0.005); Agaricaceae (11.2% in A and 11.85% in B), Lyophyllaceae (6.4% in A and 5.6% in B; p value < 0.005), Saccharomycetaceae (2.3% in A and 2.40% in B; p value < 0.005) as the topmost families and Auricularia (41% in A and 42.26% in B; p value < 0.005), Clitopilus (11.8% in A and 12.45% in B; p value < 0.005), Agaricus (6.3% in A and 6.5% in B; p value < 0.005), Termitomyces (5.6% in A and 6.4% in B;

p value < 0.005), Leucoagaricus (2.4% in A and 2.93% in B; p value < 0.005) genera.

The abundant fungal species observed were *Auricularia polytricha* (41% in A and 42.26% in B; p value < 0.005), followed by *Saccharomyces cerevisiae* (2.30% in both A and B), *Echinoderma echinacea* (1.1% in A and 0.93% in B; p value < 0.005), *Trichoderma asperellum* (0.3% in A and 0.23% in B; p value < 0.005). *Cochliobolus lunatus* (0.01%) and *Pichia kudriavzevii* (0.08%) were observed as minor species exclusively in A and B. The distribution and abundance of OTU at different taxonomic levels in A and B was plotted using heatmap where each row corresponded to an OTU and each column corresponded to the sample. The higher the relative abundance of an OTU in a sample, the more intense the color at the corresponding position in the heatmap (Fig. 1). These findings are in accordance with findings on other mangrove sediments using metagenomic approach (Simoes et al. 2015).

The diversity of the samples was studied using α -diversity or within-sample diversity and β -diversity or between-sample diversity using the OTUs. The observed species and Chao1 richness estimators calculated on the basis of abundance of the OTUs were low for A (614 and 714.7) as compared to B (665 and 771.2) indicating the latter to be comparatively rich in fungal species. This was also found to be the case with bacterial diversity determined by metagenomic studies using Illumina sequencing (Haldar and Nazareth 2018). The lower diversity of the mangrove vegetation bordering the Mandovi estuary at the given site coupled with pollution, including metals, as shown by Bicholkar and Nazareth (2015) and anthropogenic activities such as water traffic, could be factors for the decrease in fungal diversity of the Mandovi mangrove sediments as compared to that of Zuari. However, Shannon diversity index, though of high value, was similar between A and B (3.4). Bray–Curtis dissimilarity index of 0.059 indicated that although the two sediments shared common fungal communities, the proportion of the species varied between A and B. The shallow gradient for rank-abundance curves indicates an even distribution of fungal taxa in both the estuarine mangrove sediments (Fig. 2a). The plateauing of the rarefaction curves obtained from both A and B indicated that the generated sequence reads and determined OTUs were sufficient to capture the fungal diversity of these two samples (Fig. 2b).

Taken together, the study identified similar composition with regard to phyla in the sediments, but with significant difference in the abundance (p value < 0.005). Basidiomycota and Ascomycota were the two major phyla which were sub-divided into eighteen classes, families, orders, genera and species and one unassigned group in both the sediments. The top five classes observed were Agaricomycetes, Sordariomycetes, Saccharomycetes, Dothideomycetes and Eurotiomycetes. The diversity analysis based

	B.Zuari	A.Mandovi	OTU ID
Consensus Lineage			
k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Hypocreales;f_unidentified;g_unidentified	20	1447	denovo4
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Auriculariales;f_Auriculariaceae;g_Auricularia;s_Auricularia_polytricha	43721	238553	denovo21
k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Pleosporales;f_Venturiaceae;g_Fusicladium	823	812	denovo74
k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Hypocreales;f_Nectriaceae;g_Fusarium	5448	8179	denovo85
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Cortinariaceae;g_Gymnopilus	12344	9950	denovo103
k_Fungi;p_Ascomycota;c_Eurotiomycetes;o_Eurotiales;f_Trichocomaceae;g_Penicillium	3234	3041	denovo174
k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Hypocreales;f_Hypocreaceae;g_Trichoderma;s_Trichoderma_asperellum	1349	1867	denovo250
k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Capnoidiales;f_Mycosphaerellaceae;g_Guignardia	2590	88	denovo329
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Agaricaceae;g_Leucoagaricus	12374	19102	denovo421
k_Fungi;p_unidentified;c_unidentified;o_unidentified;f_unidentified;g_unidentified;s_unculturedfungus	716	833	denovo591
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Polyporales;f_Polyporaceae	972	525	denovo702
Unassigned	1683	2709	denovo997
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Agaricaceae;g_Echinoderma;s_Echinoderma_echinacea	5371	5154	denovo1041
k_Fungi;p_Ascomycota	1430	285	denovo1156
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Agaricaceae;g_Agaricus	5599	5193	denovo1276
k_Fungi;p_Basidiomycota	28279	30257	denovo1287
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Marasmiaceae;g_Marasmius	3872	2329	denovo1296
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Lyophyllaceae;g_Termitomyces	32129	34634	denovo1434
k_Fungi;p_Ascomycota;c_Saccharomycetes;o_Saccharomycetales;f_Saccharomycetaceae;g_Saccharomyces;s_Saccharomyces_cerevisiae	13599	13071	denovo1847
k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Botryosphaeriales;f_Botryosphaeriaceae;g_Lasioidiplodia	933	233	denovo2168
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Agaricaceae;g_Leucoagaricus	4014	3553	denovo2446
Unassigned	3869	4018	denovo2471
Unassigned	22984	21543	denovo2762
k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Hypocreales;f_Hypocreaceae;g_Hypocrea;s_Hypocrea_lixii	245	1044	denovo2783
Unassigned	1143	401	denovo2954
Unassigned	26671	23271	denovo3044
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Agaricaceae	8547	8120	denovo3123
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Psathyrellaceae	5704	3554	denovo3340
k_Fungi;p_unidentified;c_unidentified;o_unidentified;f_unidentified;g_unidentified	308	733	denovo3426
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Agaricaceae;g_Agaricus	31633	29997	denovo3555
k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Agaricales;f_Entolomataceae;g_Clitopilus	72045	67493	denovo3602
k_Fungi;p_Ascomycota;c_Dothideomycetes;o_Pleosporales;f_Venturiaceae;g_Venturia	5335	5253	denovo3609
k_Fungi;p_Ascomycota;c_Sordariomycetes;o_Xylariales	200	827	denovo3655
Unassigned	1633	2778	denovo3694

Fig. 1 OTU table heatmap showing taxonomy assignment for each OTU at 1000 sequences per OTU. The OTU heatmap displays raw OTU counts per sample, where the counts are colored based on the

contribution of each OTU to the total OTU count present in that sample (blue: contributes low percentage of OTUs; red: contributes high percentage of OTUs)

Fig. 2 **a** Rank-abundance and **b** rarefaction curves obtained from the Illumina sequence reads for Mandovi (A) and Zuari (B) mangrove sediments

on the observed fungal species revealed that Zuari sediment was taxonomically richer. It is noteworthy to mention that these groups of fungi have been reported to be potent candidates used for bioremediation. Hence, this

study indicated the mangrove sediments of Goa to be a rich repository for fungi with potential bioremediation abilities. This study is the first report on diversity of fungi, particularly with respect to the given classes of fungi, from

mangrove sediments of Goa by a metagenomic approach and using Illumina sequencing.

Accession number(s) The raw data for Mandovi river mangrove sediment sample have been deposited in the NCBI Sequence Read Archive (SRA) under accession number SRX3203327 and of Zauri river mangrove sediment under accession number SRX3203326.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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